<110> Korea Research Institute of Bioscience and Biotechnology

<120> Method for screening of a lipase having improved enzymatic activity using yeast surface display vector and the lipase

<130> 26666U

<150> KR 2002-55575
<151> 2002-09-13

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<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> CALB primer 1

<400> 1
ggctcttcag ccactccttt ggtgaag 27

<210> 2
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> CALB primer 2

<400> 2
gcggatcctc agggggtgac gat 23

<210> 3
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> CALB primer 3

<400> 3
gcggatccgg gggtgacgat gccggag 27

<210> 4
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> GPD-err primer

<400> 4
gcagagctaa ccaataagg 19

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<211> 19
<212> DNA
<213> Artificial Sequence

<220>

<223> T-0 primer

<400> 5
tgcagttgaa cacaaccac 19

<210> 6
<211> 1023
<212> DNA
<213> Candida antarctica

<220>
<221> sig_peptide
<222> (1)..(51)
<223> secretion signal

<400> 6
atgaatatat tttacatatt tttgtttttg ctgtcattcg ttcaaggtac cgccactccc 60

ttggtgaagc gtctgccttc cggttcggac cctgcctttt cgcagcccaa gtcggtgctc 120

gatgcgggtc tgacctgcca gggtgcttcg ccatcctcgg tctccaaacc catccttctc 180

gtccccggaa ccggcaccac aggtccacag tcgttcgact cgaactggat ccccctctct 240

gcgcagctgg gttacacacc ctgctggatc tcacccccgc cgttcatgct caacgacacc 300

caggtcaaca cggagtacat ggtcaacgcc atcaccacgc tctacgctgg ttcgggcaac 360

aacaagcttc ccgtgctcac ctggtcccag ggtggtctgg ttgcacagtg gggtctgacc 420

ttcttcccca gtatcaggtc caaggtcgat cgacttatgg cctttgcgcc cgactacaag 480

ggcaccgtcc tcgccggccc tctcgatgca ctcgcggtta gtgcaccctc cgtatggcag 540

caaaccaccg gttcggcact cactaccgca ctccgaaacg caggtggtct gacccagatc 600

gtgcccacca ccaacctcta ctcggcgacc gacgagatcg ttcagcctca ggtgtccaac 660

tcgccactcg actcatccta cctcttcaac gggaagaacg tccaggcaca ggctgtgtgt 720

gggccgctgt tcgtcatcga ccatgcaggc tcgctcacct cgcagttctc ctacgtcgtc 780

ggtcgatccg ccctgcgctc caccacgggc caggctcgta gtgcagacta tggcattacc 840

gactgcaacc ctcttcccgc caatgatctg actcccgagc aaaaggtcgc cgcggctgcg 900

ctcccggcgc cggcggctgc agccatcgtg gcgggtccaa agcagaactg cgagcccgac 960

ctcatgccct acgcccgccc ctttgcagta ggcaaaagga cctgctccgg catcgtcacc 1020

ccc 1023

<210> 7
<211> 1023
<212> DNA
<213> Candida antarctica

<220>
<221> sig_peptide
<222> (1)..(51)
<223> secretion signal

<400> 7
atgaatatat tttacatatt tttgtttttg ctgtcattcg ttcaaggtac cgccactcct 60

ttggtgaagc gtctgccttc cggttcggac cctgcctttt cgcagcccaa gtcggtgctc 120

gatgcgggtc tgacctgcca aggtgcttcg ccatcctcgg tctccaaacc catccttctc 180

gtccccggaa ccggcaccac aggtccacag tcgttcgact cgaactggat ccccctctct 240

gcgcagctgg gttacacacc ctgctggatc tcacccccgc cgttcatgct caacgacacc 300

caggtcaaca cggagtacat ggtcaacgcc atcaccacgc tctacgctgg ttcgggcaac 360

aacaagcttc ccgtgctcac ctggtcccag ggtggtctgg ttgcacagtg gggtctgacc 420

ttcttcccca gtatcaggtc caaggtcgat cgacttatgg cctttgcgcc cgactacaag 480

ggcaccgtcc tcgccggccc tctcgatgca ctcgcggtta gtgcaccctc cgtatggcag 540

caaaccaccg gttcggcact cactaccgca ctccgaaacg caggtggtct gacccagatc 600

gtgcccacca ccaacctcta ctcggcgacc gacgagatcg ttcagcctca ggtgtccaac 660

tcgccactcg actcatccta ccttttcaac ggaaagaacg tccaggcaca ggctgtgtgt 720

gggccgcagt tcgtcatcga ccatgcaggc tcgctcacct cgcagttctc ctacgtcgtc 780

ggtcgatccg ccctgcgctc caccacgggc caggctcgta gtgcggacta tggcattacg 840

gactgcaacc ctcttcccgc caatgatctg actcccgagc aaaaggtcgc cgcggctgcg 900

ctcccggcgc cggcggctgc agccatcgtg gcgggtccaa agcagaactg cgagcccgac 960

ctcatgccct acgcccgccc ctttgcagta ggcaaaagga cctgctccgg catcgtcacc 1020

ccc 1023

<210> 8
<211> 1023
<212> DNA

<213> Candida antarctica

<220>
<221> sig_peptide
<222> (1)..(51)
<223> secretion signal

<400> 8
atgaatatat tttacatatt tttgtttttg ctgtcattcg ttcaaggtac cgccactcct 60

ttggtgaagc gtctgccttc cggttcggac cctgcctttt cgcagcccaa gtcggtgctc 120

gatgcgggtc tgacctgcca gggtgcttcg ccatcctcgg tctccaaacc catccttctc 180

gtccccggaa ccggcaccac aggtccacag tcgttcgact cgaactggat ccccctctct 240

gcgcagctgg gttacacacc ctgctggatc tcacccccgc cgttcatgct caacgacacc 300

caggtcaaca cggagtacat ggtcaacgcc atcaccacgc tctacgctgg ttcgggcaac 360

aacaagcttc ccgtgctcac ctggtcccag ggtggtctgg ttgcacagtg gggtctgacc 420

ttcttcccca gtatcaggtc caaggtcgat cgacttatgg cctttgcgcc cgactacaag 480

ggcaccgtcc tcgccggccc tctcgatgca ctcgcggtta gtgcaccctc cgtatggcag 540

caaaccaccg gttcggcact cactaccgca ctccgaaacg caggtggtct gacccagatc 600

gtgcccacca ccaacctcta ctcggcgacc gacgagatcg ttcagcctca ggtgtccaac 660

tcgccactcg actcatccta cctcttcaac ggaaagaacg tccaggcaca ggctgtgtgt 720

gggccgcagt tcgtcatcga ccatgcaggc tcgctcacct cgcagttctc ctacgtcgtc 780

ggtcgatccg ccctgcgctc caccacgggc caggctcgta gtgcagacta tggcattacg 840

gactgcaacc ctcttcccgc caatgatctg actcccgagc aaaaggtcgc cgcggctgcg 900

ctcctggcgc cggcggctgc agccatcgtg gcgggtccaa agcagaactg cgagcccgac 960

ctcatgccct acgcccgccc ctttgcagta ggcaaaagga cctgctccgg catcgtcacc 1020

ccc 1023

<210> 9
<211> 343
<212> PRT
<213> Candida antarctica

<220>
<221> SIGNAL
<222> (1)..(17)
<223> secretion signal

<400> 9

Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly
1 5 10 15

Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala
20 25 30

Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly
35 40 45

Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr
50 55 60

Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser
65 70 75 80

Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met
85 90 95

Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr
100 105 110

Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp
115 120 125

Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser
130 135 140

Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys
145 150 155 160

Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro
165 170 175

Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg
180 185 190

Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser
195 200 205

Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp
210 215 220

Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys
225 230 235 240

Gly Pro Leu Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe
245 250 255

Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala
260 265 270

Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn
275 280 285

Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Ala Leu Pro Ala Pro
290 295 300

Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp
305 310 315 320

Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser
325 330 335

Gly Ile Val Thr Pro Gly Ser
340

<210> 10
<211> 343
<212> PRT
<213> Candida antarctica

<220>
<221> SIGNAL
<222> (1)..(17)
<223> secretion signal

<400> 10
Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly
1 5 10 15

Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala
20 25 30

Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly

35 40 45

Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr
50 55 60

Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser
65 70 75 80

Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met
85 90 95

Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr
100 105 110

Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp
115 120 125

Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser
130 135 140

Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys
145 150 155 160

Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro
165 170 175

Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg
180 185 190

Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser
195 200 205

Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp
210 215 220

Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys

225 230 235 240

Gly Pro Gln Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe
245 250 255

Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala
260 265 270

Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn
275 280 285

Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Ala Leu Pro Ala Pro
290 295 300

Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp
305 310 315 320

Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser
325 330 335

Gly Ile Val Thr Pro Gly Ser
340

<210> 11
<211> 341
<212> PRT
<213> Candida antarctica

<220>
<221> SIGNAL
<222> (1)..(24)
<223> secretion signal

<400> 11

Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly
1 5 10 15

Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala
20 25 30

Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly
35 40 45

Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr
50 55 60

Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser
65 70 75 80

Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met
85 90 95

Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr
100 105 110

Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp
115 120 125

Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser
130 135 140

Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys
145 150 155 160

Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro
165 170 175

Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg
180 185 190

Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser
195 200 205

Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp
210 215 220

Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys
225 230 235 240

Gly Pro Gln Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe
245 250 255

Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala
260 265 270

Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn
275 280 285

Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Ala Leu Leu Ala Pro
290 295 300

Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp
305 310 315 320

Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser
325 330 335

Gly Ile Val Thr Pro
340

<210> 12
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> CALB primer 4

<400> 12
ctcatatgct accttccggt tcggac 26

<210> 13
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> a-amylase secretion signal

<400> 13
Met Met Val Ala Trp Trp Ser Leu Phe Leu Tyr Gly Leu Gln Val Ala
1 5 10 15

Ala Pro Ala Leu Ala
20

<210> 14
<211> 317
<212> PRT
<213> Candida antarctica

<400> 14
Leu Pro Ser Gly Ser Asp Pro Ala Phe Ser Gln Pro Lys Ser Val Leu
1 5 10 15

Asp Ala Gly Leu Thr Cys Gln Gly Ala Ser Pro Ser Ser Val Ser Lys
20 25 30

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Pro Ile Leu Leu Val Pro Gly Thr Gly Thr Thr Gly Pro Gln Ser Phe
        35                  40                  45

Asp Ser Asn Trp Ile Pro Leu Ser Ala Gln Leu Gly Tyr Thr Pro Cys
    50                  55                  60

Trp Ile Ser Pro Pro Pro Phe Met Leu Asn Asp Thr Gln Val Asn Thr
65                  70                  75                  80

Glu Tyr Met Val Asn Ala Ile Thr Thr Leu Tyr Ala Gly Ser Gly Asn
                85                  90                  95

Asn Lys Leu Pro Val Leu Thr Trp Ser Gln Gly Gly Leu Val Ala Gln
            100                 105                 110

Trp Gly Leu Thr Phe Phe Pro Ser Ile Arg Ser Lys Val Asp Arg Leu
        115                 120                 125

Met Ala Phe Ala Pro Asp Tyr Lys Gly Thr Val Leu Ala Gly Pro Leu
    130                 135                 140

Asp Ala Leu Ala Val Ser Ala Pro Ser Val Trp Gln Gln Thr Thr Gly
145                 150                 155                 160

Ser Ala Leu Thr Thr Ala Leu Arg Asn Ala Gly Gly Leu Thr Gln Ile
                165                 170                 175

Val Pro Thr Thr Asn Leu Tyr Ser Ala Thr Asp Glu Ile Val Gln Pro
            180                 185                 190

Gln Val Ser Asn Ser Pro Leu Asp Ser Ser Tyr Leu Phe Asn Gly Lys
        195                 200                 205

Asn Val Gln Ala Gln Ala Val Cys Gly Pro Leu Phe Val Ile Asp His
    210                 215                 220
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Ala Gly Ser Leu Thr Ser Gln Phe Ser Tyr Val Val Gly Arg Ser Ala
225 230 235 240

Leu Arg Ser Thr Thr Gly Gln Ala Arg Ser Ala Asp Tyr Gly Ile Thr
245 250 255

Asp Cys Asn Pro Leu Pro Ala Asn Asp Leu Thr Pro Glu Gln Lys Val
260 265 270

Ala Ala Ala Ala Leu Leu Ala Pro Ala Ala Ala Ala Ile Val Ala Gly
275 280 285

Pro Lys Gln Asn Cys Glu Pro Asp Leu Met Pro Tyr Ala Arg Pro Phe
290 295 300

Ala Val Gly Lys Arg Thr Cys Ser Gly Ile Val Thr Pro
305 310 315

<210> 15
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> LQ53 primer

<400> 15
gctgtgtgtg ggccgcagtt cgtcatcg 28

<210> 16
<211> 30
<212> DNA

<213> Artificial Sequence

<220>
<223> LQ35 primer

<400> 16
gcatggtcga tgacgaactg cggcccacac 30

<210> 17
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> LP53 primer

<400> 17
gtcgccgcgg ctgcgctccc ggcgccggcg 30

<210> 18
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> LP35 primer

<400> 18
ctgcagccgc cggcgccggg agcgcagcc 29

<210> 19

<211> 343

<212> PRT

<213> Candida antarctica

<400> 19

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Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly
 1               5                  10                  15

Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala
            20                  25                  30

Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly
        35                  40                  45

Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr
    50                  55                  60

Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser
65                  70                  75                  80

Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met
                85                  90                  95

Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr
            100                 105                 110

Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp
        115                 120                 125

Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser
    130                 135                 140

Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys
145                 150                 155                 160
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Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro
165 170 175

Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg
180 185 190

Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser
195 200 205

Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp
210 215 220

Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys
225 230 235 240

Gly Pro Leu Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe
245 250 255

Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala
260 265 270

Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn
275 280 285

Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Ala Leu Leu Ala Pro
290 295 300

Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp
305 310 315 320

Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser
325 330 335

Gly Ile Val Thr Pro Gly Ser
340